General discussion

9

"Gut health": the new objective in medicine

In general, gut health can be defined as a state of physical and mental wellbeing in the absence of gastrointestinal (GI) symptoms.[1,2] However, this definition does not cover the multiple aspects that are linked to gut health such as effective digestion and immune status.[2] In this thesis different aspects of gut health were investigated, among other methods, by comparing characteristics of individuals without GI symptoms with those of individuals with a range of GI symptoms including irritable bowel syndrome (IBS) patients as a group with severe GI symptoms. IBS is a functional gastrointestinal disorder prevalent in 9-15% of the North European population that is characterized by altered bowel movements and symptoms of abdominal discomfort or pain.[3,4] In previous chapters of this thesis, we learned about the factors associated with gut microbiota composition, a panel of biomarkers to measure gut health, differences in dietary intake between individuals with and without GI symptoms, the effect of reduced gut health on quality of life (QoL) and the importance of large scale follow-up studies to study the complex interactions between the different aspects of gut health. Our knowledge about gut health is enriched by the results described in this thesis, however the inconclusive definition of gut health and the fact that it includes multiple domains continues to make research into gut health and the interpretation of its results challenging. In this final chapter I focus on several aspects of gut health in the extension of the findings described in previous chapters, including gastrointestinal symptoms, biomarkers, gut microbiota, mental health and food aspects, and make recommendations for future research.

Gastrointestinal symptoms and gut health

Presence of gastrointestinal symptoms is currently widely used in the diagnosis of gastrointestinal disorders. After exclusion of organic gastrointestinal disease, the diagnosis of functional gastrointestinal disorders depends on the type and level of GI symptoms. However, GI symptoms as a measure of gut health cannot be measured objectively. The first criteria for the diagnosis of IBS based on GI symptoms was reported by Manning in 1978, and included criteria related to abdominal pain and distension.[5] Ten years later, in preparation for the 1988 International Congress of Gastroenterology in Rome, a working team produced guidelines for the management and study of IBS.[6] In 1990, the Rome diagnostic criteria were broadened from IBS to include functional gastrointestinal disorders (FGIDs) encompassing 21 entities in five anatomical regions of the gut.[7] Since then, the Rome foundation has published three revisions of the criteria for IBS and FGIDs.[8] Recently, the fourth update of these criteria (the ROME-IV criteria) was released.[9] Despite the consensus on the criteria for the definition of IBS, diagnosis remains largely subjective as it depends mostly on a subject's own perception of symptoms. Recently, Mujagic et al. showed differences in GI symptom perception by comparing end-of-day GI symptom scores to the mean of scores taken at ten random time points during the day.[10] They found the end-of-day GI symptoms scores were higher than the mean of the scores taken at multiple time points, which illustrates the recall bias in reporting GI symptoms. The survey method can also influence the

outcome. A self-administered questionnaire was found to lead to higher IBS prevalence rates compared to a personal interview.[11] We saw this effect in our LifeLines DEEP (LLD) cohort in the high prevalence of IBS (21%) diagnosed via self-administered questionnaire (Chapter 1)[12] and in the difference in severity of GI symptoms in these population-based IBS patients (LLD) versus the clinical IBS patients (MIBS cohort) diagnosed via personal interview by the gastroenterologist (Chapter 8)[13]. One could speculate that the self-administered questionnaire leads to a diagnosis based on milder symptoms compared to a diagnosis based on more severe symptoms in personal interview. Objective measurement of gastrointestinal symptoms is not as yet possible, although more accurate measurement of symptoms, including severity and fluctuations over the course of the day, might be achieved via daily measurements at multiple time points via, for example, a smartphone app.[10,14] Previous studies in IBS patients have found compliance rates between 77% and 88% for multiple-time-point assessment of GI symptoms via an electronic device.[10,14] There is, in general, a bright future for more accurate data collection through smartphone apps as more people are now used to working with the device and are more willing to share their data, and apps provide immense health-monitoring opportunities that can be developed for different target populations.[15,16]

Biomarker view on gut health

Viewing the gastrointestinal system as an organ, its physiology, including its immune system and barrier function, has been used as potential source of biomarkers. A biomarker, or biological marker, is defined as an indicator of a physiological or pathological state that can be objectively measured and evaluated. It should be simple and easy to use, non-invasive and reproducible, i.e. the intra- and inter-individual variability should be low.[17] Thus far, the search for biomarkers for gut health has mainly focused on single biomarkers, e.g. calprotectin for gastrointestinal diseases like inflammatory bowel disease (IBD).[18,19] Interest has now shifted from single biomarkers to multiple biomarker panels for prediction of GI disease. Some panels have used serum biomarkers or a combination of gene expression and serology markers[20] as a diagnostic test for IBS.[21] Other panels have focused on plasma amino acid profiles[22] or fecal markers of inflammation[17] for IBD. Another panel also included clinical tools for the assessment of gut function.[23] In 2015, Camilleri published a review of biomarkers in FGIDs, but concluded that no biomarkers were ready to be included as diagnostic tests for IBS and that future studies should focus on plasma serotonin levels, gut inflammatory state, colonic transit and bile acid malabsorption as relevant targets for identification of subgroups of patients with specific gastrointestinal symptoms.[24] This conclusion illustrates that studying physiological aspects of the gut alone might not be the key to finding reliable markers for gut health and that a more systemic approach is needed.

During the biomarker selection process in the first phase of this thesis project, we took this into account by making sure to select biomarkers representing Bischoff's five major aspects of gut health.[2] For example, using fecal human-β-defensin-2 as a marker for defense against invading

microbes and plasma citrulline as a measure for enterocyte mass. What we observed was that our integrated biomarker panel that included biomarkers from four out of five domains (sensitivity 88.1% and specificity 86.5%) outperformed most single or serum biomarkers (sensitivity 50.0-82.0% and specificity 64.0-90.0%)[25] in discriminating between IBS patients and healthy controls (Chapter 8)[13]. We thus showed the importance of a more systemic approach in assessment of gut health.

Another potentially powerful approach for examining gut health is the use of the volatile organic compound (VOC) profile of exhaled air (i.e. breathomics) as a reflection of the body's metabolic state because the occurrence of chronic inflammation and/or oxidative stress can result in excretion of volatile compounds that generate a unique VOC pattern.[26] We therefore collected exhaled air samples from our study participants at the same time point as fecal sample and blood sample collection, and established a panel of 16 exhaled air VOCs to discriminate between IBS patients and healthy controls.[27] Moreover, this panel has potential to be used for the assessment of gut health since it correlated with gastrointestinal symptoms. Potential confounding factors that should be taken into account in analysis of breath samples are fiber intake[28] and smoking habits[29,30]. However, if these confounding factors are taken into account, for example via implementation of standard operating procedures, integrated breathomics devices such as e-noses provide output that has a high potential for use as biomarkers because these devices are non-invasive and can be produced at low cost.

VOCs can also be measured from fecal samples. This could be of particular interest in gut health research since the VOCs emitted from feces represent a combination of the effects of nutrition and digestion, health related metabolism and gut microbiota activity. Previous studies have shown differences in VOC patterns from fecal samples of patients with GI disorders including *Clostridium difficile* infection and IBS as compared to those from healthy controls (as recently reviewed by Chan et al).[31] Moreover, fecal VOCs can be measured from frozen stool samples, making it possible to measure fecal biomarkers, microbiota composition and fecal VOCs in one stool sample.

Another important aspect of biomarker research is the validation of the biomarker (panel) to test how it performs in an independent group of subjects. To facilitate the feasibility of such validation studies, large population cohorts like LifeLines are crucial. Including participants from the general population will teach us about generalizability of a biomarker, whilst following the participants over time will provide valuable information about the performance of the biomarker under changing circumstances within the same individual, e.g. aging and changing health status. We tested the validity of the biomarker panel (Chapter 8)[13] and the VOC panel[27], both developed in the Maastricht IBS cohort, in our population LLD cohort. From these studies we learned that the panels correlated well with GI symptoms in clinical IBS patients as well as in individuals with GI complaints from the general population. These findings offer opportunities for future assessment of gut health in the general population.

Gut bugs mark gut health

The gut microbiota is the heaviest aspect of gut health, since the trillions of bacteria that populate the human gut together weigh up to one kilogram.[32] Different aspects of the microbiota, like the bacterial composition and the main functions of the bacterial species, could inform us about an individual's gut health status. In general, more diversity in the microbiota composition is considered healthier. In studies of gut microbiota composition it is important to use standardized methods for fecal sample collection, transportation, storage, DNA isolation and microbiota composition analysis, as all these aspects could influence the microbiota composition measured. By using standardized methods for fecal sample collection and gut microbiota analysis in samples from more than 1000 individuals in the LifeLines DEEP cohort, we have identified 126 factors that influence microbiota composition and diversity, which together explain about 19% of microbiota variation.[12,33–35] Broadly, dietary fibers and polyphenols, age and high-density lipoprotein cholesterol are associated with higher diversity, whilst high fat and sugar intake, chromogranin A (CgA) level, disease and medication are associated with lower diversity. However, high microbiota diversity does not necessarily imply high functional diversity. We saw this in our gluten-free diet (GFD) intervention study in which we observed increased bacterial functional activities, where the diversity (number of different bacteria) did not change but the microbiota composition (percentage of one group of bacteria versus another) did.[33] These findings support the idea that different bacteria can execute a particular function such as digestion of starch.[36] Nevertheless, the analysis of gut bacterial composition and bacterial functions are mainly based on incomplete, though regularly updated, databases, and we are just beginning to understand the potential of gut bacteria. There is still a need to unravel the functional consequences of interactions between microbiota and environmental or genetic factors. Recently, we described how genetics and genetic variation in innate immunity, the genes involved in metabolism and food molecules could shape the gut.[37] We, for example, identified a strong association between a large intergenic region on chromosome 9 and the abundance of bacteria from the *Blautia* genus.

Identification of a microbiome signature for a healthy gut would provide opportunities for improved diagnosis and treatment of microbiota- and gut-health-related-diseases. Thus far, no comprehensive microbiota signature for IBS has been established because of differences between studies and variability between the subtypes of IBS. As an example of this variability, in IBS patients overall the abundance of Bacteroidetes was decreased but was increased in IBS patients with predominant symptoms of diarrhea (IBS-D) compared to controls.[38] Nevertheless, our work did enhance our understanding of the microbiota and provided a first mapping of the important factors that interact with gut microbiota composition. A better understanding of the gut bacteria's functions is needed to disentangle potential therapeutic targets to improve gut health.

Mental health and gut health (happy gut = happy person)

One of the intriguing factors of gut health that personally fascinates me is its relation to brain function – the gut-brain axis (GBA). This communication system between the central and generic nervous system links emotional and cognitive centers of the brain with intestinal functions. We see this reflected in both cohorts studied in this thesis in that we observe significantly lower scores for quality of life in patients with GI symptoms ranging from mild to severe (Chapter 7), as well as higher anxiety and depression scores in clinical IBS patients versus controls from the MIBS cohort (Chapter 6).

The mechanisms by which the gut microbiota exert an effect on the brain include the production of neurotransmitters (e.g. serotonin) and bacterial metabolites (e.g. short chain fatty acids), protection of the intestinal barrier, modulation of sensory nerves and mucosal immune regulation.[39] Reciprocally, the brain also has the capacity to influence the gut via alterations in mucus production, motility, intestinal permeability and immune function.[39] Elaborating on this involvement of the GBA in IBS, Mayer et al. described a model of IBS pathophysiology in which (epi)genetic factors influence the brain and gut, whose intermediate phenotypes interact with each other. When the balance of these factors is disturbed, normal gastrointestinal symptoms start to be consciously perceived, and this results in clinical IBS.[40] The recent advances in –omics technologies provide the opportunity to study IBS from a systems biological perspective that includes changes in proteomics, transcriptomics and metabolomics.[40] As we, for example, have shown on one level by identification of a biomarker profile of VOCs in our study of breathomics.[27] Integration of this metabolomics profile from exhaled air with profiles from blood, genetics and microbiome data will further aid our understanding of gut health.

The importance of the mental component of IBS is also reflected in the new Rome criteria for FGIDs entitled: "Rome IV—Functional GI Disorders: Disorders of Gut-Brain Interaction". Because of this interaction between gut and brain, we selected CgA as a potential biomarker for gut health as it is secreted by enteric, immune and endocrine cells, and has previously been linked to IBS.[41] In our biomarker panel study, CgA was indeed selected as one of the eight out of fifteen measured biomarkers that contribute significantly to the biomarker panel for discriminating between IBS patients and healthy controls. We also confirmed previous findings of higher CgA excretion in IBS patients versus controls in both the MIBS and the LLD cohort, and discovered a strong association between fecal CgA level and microbiota composition.[13,42] The median fecal CgA levels ranged from 9.4 in healthy controls to 11.1 in a general population sample (LLD) to 15.2 in clinical IBS patients. The fact that the CgA levels in the general population were higher compared to healthy controls is consistent with these healthy controls being preselected to exclude gastrointestinal symptoms, which makes them healthier in comparison to our general population sample in which the prevalence of FGIDs was 35% (Chapter 1).[12] Moreover, CgA levels in the LLD cohort were associated with self-reported IBS (Chapter 4).[42] In our GFD study we observed that levels of CgA did not change upon dietary intervention with gluten (Chapter 5)[33], however in the large scale metagenomic analysis in 1135 subjects, we observed a negative correlation between CgA level

and intake of fruit and vegetables. Moreover, we observed positive correlations of CgA level with softer stool type and stool frequency.[42] This might link to gut transit time, as previous research showed higher CgA levels upon shorter colonic transit time.[41] Overall, fecal CgA level seems to be an important aspect of gut health since it can be linked to the microbiota, gut complaints and dietary factors.

Thinking about Bischoff's fifth criterion for gut health (overall wellbeing) and the well-known fact that individuals with GI complaints experience a reduced quality of life (confirmed in our cohorts (Chapter 7)), made us curious if, apart from health related factors, genetic factors could play a role in overall wellbeing. In our subsequent investigation, we identified 113 SNPs suggestively associated to QoL (Chapter 7.1). Interestingly, a considerable number of these SNPs (n=24) were associated to the two QoL domains 'role' that assess the extent to which the respondent experiences problems with work or other regular activities because of emotional or physical health problems. Though one cannot change their genetic background, these findings could be of guidance in treatment of individuals with GI symptoms as a factor to consider when assessing the impact of their complaints on work and daily activities. Treatment could, for example, focus on strategies to limit the impact of GI complaints on daily activities, which might eventually lead to improved QoL. Previous research showed a positive effect of cognitive behavioral therapy and hypnotherapy on QoL in IBS patients.[43,44]

The food factor of gut health

The effect of diet on gut health is intriguing, since one needs a healthy gut for effective digestion and absorption of food while, at the same time, specific food components can exert an effect on gut health. In this thesis, we saw that GI symptoms can give rise to (unfavorable) changes in food intake (Chapter 6), and we also identified food factors that either positively or negatively affected gut microbiota diversity (Chapters 4 and 5). Collection of accurate dietary intake data is important to study the interactions between food components and gut microbiota in detail. However, current methods for the assessment of dietary intake are time consuming and plagued by reporting bias.[45] Development of more efficient and accurate collection methods will be necessary to perform wide-scale longitudinal studies on food intake. Web-based (e.g. via smart phone or tablet) dietary assessment methods currently being implemented have proven to be efficient, cost-effective, less burdensome to respondents and reliable in assessment of food intake.[46,47]. Moreover, web-based applications can easily be combined with other (web-based) measurements at the same time point. Gill et al., for example, used a smart phone app to record dietary intake by having study participants photograph the foods they consumed and combined that with a wrist actimetry sensor for activity and sleep detection. This study demonstrated the value of this combination for the identification of dietary patterns, as well as its possibility to support improved dietary intake.[48] Another study used a website for data collection on food intake (food items selected from a list), sleep and physical activity over one week. This data was combined with data from continuous blood glucose monitoring, microbiota composition, blood

parameters, questionnaires and anthropometrics to provide personalized nutritional advice to improve blood glucose levels.[49] These examples show that wearable and/or portable devices could improve accurate measurement of biological and environmental factors. This relatively new area of research should be further explored for future research of gastrointestinal health.

Biomarkers for food intake should be considered as another strategy to improve the accuracy of dietary intake assessment. In 2012, Hedrick et al. systematically reviewed the literature on dietary biomarkers and described ten biomarkers related to macronutrients, e.g. fatty acids and animal protein, and five biomarkers related to other dietary components. e.g. caffeine and citrus.[50] However, application of these biomarkers into practice was considered limited, since only the biomarkers urinary sucrose and fructose for sugar content and plasma alkylresorcinol for whole grain intake met all four biomarker criteria (validity, reproducibility, ability to detect changes over time and suitability for measurement in the general population). Beyond this single biomarker approach, food metabolomics, i.e. all metabolites directly derived from digestion and metabolism of food chemicals, have also been the focus of increasing interest in the search for biomarker profiles for food intake. An overview of previously identified metabolomic profiles for 40 dietary factors such as vegetables and salmon, obtained from urine, plasma or serum, is provided in the review of Scalbert et al.[51] However, standardized procedures, reference databases and algorithms for data-analysis are still needed to support future food metabolomics research and eventually make these food metabolomics profiles suitable for use in practice. Nevertheless, biomaterial for measurement of food metabolomics will be relatively readily available in large cohort studies like LifeLines because blood and urine are collected on a regular basis. Food metabolomics would thus be an important factor to take into account in future assessment of gut health.

TABLE 1 | Schematic overview of adherence to biomarker criteria ranging from very bad (--) to excellent (+++) for different biomarkers of gut health

Biomarker	Simple*	Reliable**	Reproducible***	Non-invasive	Costs	Conclusion
Single from blood	-	-+	-	--	€	☹
Single from feces	++	-+	-	+	€	😐
Panel from blood	-	+	-+	--	€€	😐
Panel from feces	++	+	-+	+	€€	😐
Panel combined blood and fecal markers	-+	+	+	-+	€€€	☺
VOCs profile from exhaled air	++	+	-+	++	€€	☺
Microbiota profile	++	+	++	+	€€	☺
Combined VOCs and microbiota profile	+	++	-+	+	€€€	☺☺
Combined microbiota profile, blood and fecal markers	-+	++	++	-+	€€€	☺☺
Combined VOCs and microbiota profile, blood and fecal markers, and food intake markers	+	+++	+++	+	€€€€	☺☺☺

* simple also includes suitability for measurement in the general population, ** reliable also includes validity, i.e. objective measurement and evaluation, *** reproducible also includes detection of changes over time

Future perspectives

In summary, this thesis enriches our knowledge about different aspects of gut health ranging from bugs (microbiota analysis) to buttermilk (food intake), yet new questions have also arisen over the course of this research. The multifactorial nature of gut health challenges future researchers to think about new/improved methods for the analysis of gut health. It is evident that single biomarkers fail to fulfill the main biomarker criteria (simple, reliable, reproducible and non-invasive) (Table 1). In comparison, a panel of biomarkers has an improved biomarker performance and, as shown in this thesis, measurements from feces are simple and non-invasive. It is moreover recommended that measurements from different biomaterials be combined to better cover the broad spectrum of gut health domains (Table 1). Our findings on recent advances in microbiomics and breathomics further highlight the biomarker potential of these –omics techniques, as the integration of multiple aspects into one –omics profile increases reliability and reproducibility. At present, the highest potential for a biomarker for gut health would be one that integrates the different aspects of gut health measured systemically (i.e. –omics) with more local measures (i.e. biomarkers from feces and food intake). Future research should focus on this integration of different levels. There is also a need for better registration methods for gastrointestinal symptoms that take into account daily as well as day-to-day fluctuations. Ideally, food intake and psychological status should be assessed at the same time. Moreover, large scale microbiota studies with multiple follow-up moments are needed to further study the functional consequences of microbiota interaction with environmental and genetic factors. The work in this thesis supports the potential for large scale fecal sample collections, as it shows the willingness of participants to collect samples at home and store them in their home freezer to optimally preserve sample quality. A fifth important consideration for biomarker research is the cost of measurement (Table 1). The cost is still quite high because relatively new techniques are used while other techniques still need to be developed. However, as we have seen in GWAS analysis, improved techniques are often accompanied by reduced analysis costs. Further, the introduction of good gut health measurements will help decrease GI symptoms which will ultimately decrease societal costs by reducing work absenteeism and use of health care facilities.

Ultimately, the assessment of gut health via a "gutomics" profile (including data on microbiota, fecal and blood biomarkers, gastrointestinal symptoms, food intake and psychological status) should become available to everybody. This profile would help to deliver personal advice to the individual about gut health state including tips for improvement. This assessment should be non-invasive, easy to perform and log the acquired data to provide the opportunity for regular monitoring, e.g. via a portable device similar to a pedometer connected to a smartphone (Figure 1). Future research should contribute to the development of personalized probiotics and nutrition tuned to an individual's microbiota, genetic make-up and lifestyle.

To conclude, gastrointestinal health is an important health parameter that deserves a good assessment method to aid the development of therapeutic targets for improvement of gastrointestinal health and overall quality of life.

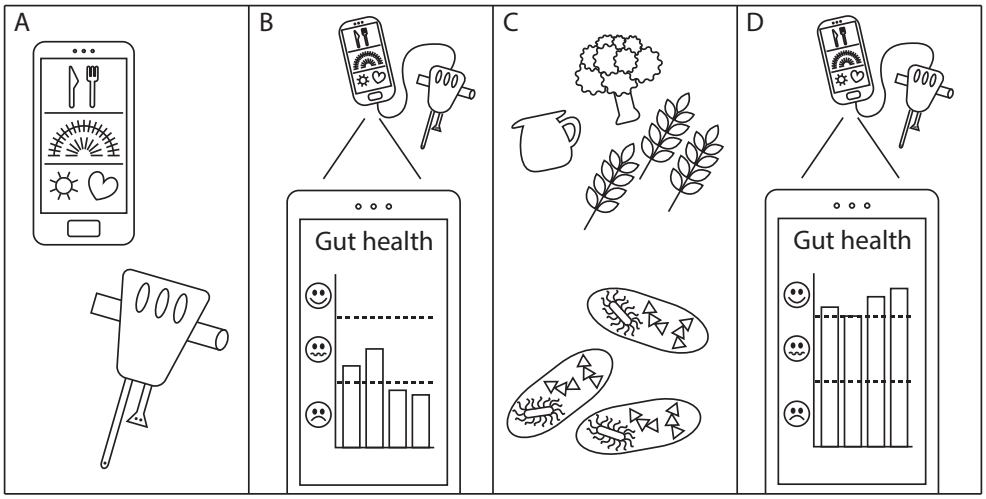

FIGURE 1 | A model for future gut health monitoring and supplementation. (A) Data collection on food intake, gastrointestinal complaints and wellbeing via smart phone, and collection of volatile organic compounds from feces and air, microbiota and biomarker analysis via electronic device. (B) Gut health scores can be followed through Apps on mobile phones for example. (C) Suggestions for personalized administration of dietary advice and supplement with specific beneficial bacteria. (D) The App will show changes after follow-up measurement.

References

1. *Preamble to the Constitution of the World Health Organization as Adopted by the International Health Conference*. New York; 1948.
2. Bischoff SC. "Gut health": a new objective in medicine? *BMC Med*. 2011;9:24. doi:10.1186/1741-7015-9-24.
3. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology*. 2006;130(5):1480-1491. doi:10.1053/j.gastro.2005.11.061.
4. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol*. 2012;10(7):712-721.e4. doi:10.1016/j.cgh.2012.02.029.
5. Manning AP, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel. *Br Med J*. 1978;2(6138):653-654.
6. Thompson W, Dotevall G, Drossman D, Heaton K, Kruis W. Irritable bowel syndrome: guidelines for the diagnosis. *Gastroenterol Int*. 1989;2:92-95.
7. Drossman D, Funch-Jensen P, Janssens J, Talley N, Thompson W, Whitehead W. Identification of subgroups of functional bowel disorders. *Gastroenterol Int*. 1990;3:159-172.
8. Thompson WG. The road to rome. *Gastroenterology*. 2006;130(5):1552-1556. doi:10.1053/j.gastro.2006.03.011.
9. Lacy BE, Mearin F, Chang L, et al. Bowel Disorders. *Gastroenterology*. 2016;150(6):1393-1407.e5. doi:10.1053/j.gastro.2016.02.031.
10. Mujagic Z, Leue C, Vork L, et al. The Experience Sampling Method--a new digital tool for momentary symptom assessment in IBS: an exploratory study. *Neurogastroenterol Motil*. 2015;27(9):1295-1302. doi:10.1111/nmo.12624.
11. Sperber AD, Dumitrascu D, Fukudo S, et al. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review. *Gut*. 2016. doi:10.1136/gutjnl-2015-311240.
12. Tigchelaar EF, Zhernakova A, Dekens JAM, et al. Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. *BMJ Open*. 2015;5(8):e006772. doi:10.1136/bmjopen-2014-006772.
13. Mujagic Z, Tigchelaar EF, Zhernakova A, et al. A novel biomarker panel for irritable bowel syndrome and the application in the general population. *Sci Rep*. 2016;6:26420. doi:10.1038/srep26420.
14. Zia J, Schroeder J, Munson S, et al. Feasibility and Usability Pilot Study of a Novel Irritable Bowel Syndrome Food and Gastrointestinal Symptom Journal Smartphone App. *Clin Transl Gastroenterol*. 2016;7:e147. doi:10.1038/ctg.2016.9.
15. Sonck N, Fernee H. *Using Smartphones in Survey Research: A Multifunctional Tool*. The Hague; 2013.
16. Chen J, Bauman A, Allman-Farinelli M. A Study to Determine the Most Popular Lifestyle Smartphone Applications and Willingness of the Public to Share Their Personal Data for Health Research. *Telemed J E Health*. 2016. doi:10.1089/tmj.2015.0159.
17. Judd TA, Day AS, Lemberg DA, Turner D, Leach ST. Update of fecal markers of inflammation in inflammatory bowel disease. *J Gastroenterol Hepatol*. 2011;26(10):1493-1499. 10.1111/j.1440-1746.2011.06846.x.
18. Mao R, Xiao YL, Gao X, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies. *Inflamm Bowel Dis*. 2012. doi:10.1002/ibd.22861.
19. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis*. 2012. doi: 10.1002/ibd.22917.
20. Jones MP, Chey WD, Singh S, et al. A biomarker panel and psychological morbidity differentiates the irritable bowel syndrome from health and provides novel pathophysiological leads. *Aliment Pharmacol Ther*. 2014;39(4):426-437. doi:10.1111/apt.12608.
21. Lembo AJ, Neri B, Tolley J, Barken D, Carroll S, Pan H. Use of serum biomarkers in a diagnostic test for irritable bowel syndrome. *Aliment Pharmacol Ther*. 2009;29(8):834-842. doi:10.1111/j.1365-2036.2009.03975.x.
22. Hisamatsu T, Okamoto S, Hashimoto M, et al. Novel, objective, multivariate biomarkers composed of plasma amino acid profiles for the diagnosis and assessment of inflammatory bowel disease. *PLoS One*. 2012;7(1):e31131. doi:10.1371/journal.pone.0031131.

23. Spiller RC. Potential biomarkers. *Gastroenterol Clin North Am*. 2011;40(1):121-139. doi:10.1016/j.gtc.2011.01.001.
24. Camilleri M. Review article: biomarkers and personalised therapy in functional lower gastrointestinal disorders. *Aliment Pharmacol Ther*. 2015;42(7):818-828. doi:10.1111/apt.13351.
25. Sood R, Law GR, Ford AC. Diagnosis of IBS: symptoms, symptom-based criteria, biomarkers or "psychomarkers"? *Nat Rev Gastroenterol Hepatol*. 2014;11(11):683-691. doi:10.1038/nrgastro.2014.127.
26. Boots AW, van Berkel JJ, Dallinga JW, Smolinska A, Wouters EF, van Schooten FJ. The versatile use of exhaled volatile organic compounds in human health and disease. *J Breath Res*. 2012;6(2):27108. doi:10.1088/1752-7155/6/2/027108.
27. Baranska A, Mujagic Z, Smolinska A, et al. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. *Aliment Pharmacol Ther*. 2016;44(1):45-56. doi:10.1111/apt.13654.
28. Raninen KJ, Lappi JE, Mukkala ML, et al. Fiber content of diet affects exhaled breath volatiles in fasting and postprandial state in a pilot crossover study. *Nutr Res*. 2016;36(6):612-619. doi:10.1016/j.nutres.2016.02.008.
29. Gaida A, Holz O, Nell C, et al. A dual center study to compare breath volatile organic compounds from smokers and non-smokers with and without COPD. *J Breath Res*. 2016;10(2):026006. doi:10.1088/1752-7155/10/2/026006.
30. Hauschild A-C, Frisch T, Baumbach JI, Baumbach J. Carotta: Revealing Hidden Confounder Markers in Metabolic Breath Profiles. *Metabolites*. 2015;5(2):344-363. doi:10.3390/metabo5020344.
31. Chan DK, Leggett CL, Wang KK. Diagnosing gastrointestinal illnesses using fecal headspace volatile organic compounds. *World J Gastroenterol*. 2016;22(4):1639-1649. doi:10.3748/wjg.v22.i4.1639.
32. The Human Microbiome Project. The human microbiome. http://hmpdacc.org/overview/about.php. Accessed May 1, 2016.
33. Bonder MJ, Tigchelaar EF, Cai X, et al. The influence of a short-term gluten-free diet on the human gut microbiome. *Genome Med*. 2016;8(1):45. doi:10.1186/s13073-016-0295-y.
34. Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. *Gut*. 2015;65(5). doi:10.1136/gutjnl-2015-310376.
35. Fu J, Bonder MJ, Cenit MC, et al. The Gut Microbiome Contributes to a Substantial Proportion of the Variation in Blood Lipids. *Circ Res*. 2015;117(9):817-824. doi:10.1161/CIRCRESAHA.115.306807.
36. Ze X, Duncan SH, Louis P, Flint HJ. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISME J*. 2012;6(8):1535-1543. doi:10.1038/ismej.2012.4.
37. Bonder MJ, Kurilshikov A, Tigchelaar EF, et al. The effect of host genetics on the gut microbiome. *Nat Genet*. 2016. doi:10.1038/ng.3663.
38. Rajilić-Stojanović M, Jonkers DM, Salonen A, et al. Intestinal Microbiota And Diet in IBS: Causes, Consequences, or Epiphenomena? *Am J Gastroenterol*. 2015;110(September 2014):278-287. doi:10.1038/ajg.2014.427.
39. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol Q Publ Hell Soc Gastroenterol*. 2015;28(2):203-209.
40. Mayer E a., Labus JS, Tillisch K, Cole SW, Baldi P. Towards a systems view of IBS. *Nat Rev Gastroenterol Hepatol*. 2015;12:592-605. doi:10.1038/nrgastro.2015.121.
41. Ohman L, Stridsberg M, Isaksson S, Jerlstad P, Simrén M. Altered levels of fecal chromogranins and secretogranins in IBS: relevance for pathophysiology and symptoms? *Am J Gastroenterol*. 2012;107(3):440-447. doi:10.1038/ajg.2011.458.
42. Zhernakova A, Kurilshikov A, Bonder MJ, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science (80-)*. 2016;352(6285). doi:10.1126/science.aad3369.
43. Boersma K, Ljótsson B, Edebol-Carlman H, Schrooten M, Linton SJ, Brummer RJ. Exposure-based cognitive behavioral therapy for irritable bowel syndrome. A single-case experimental design across 13 subjects. *Cogn Behav Ther*. 2016:1-16. doi:10.1080/16506073.2016.1194455.
44. Shahbazi K, Solati K, Hasanpour-Dehkordi A. Comparison of Hypnotherapy and Standard Medical Treatment Alone on Quality of Life in Patients with Irritable Bowel Syndrome: A Randomized Control Trial. *JCDR*. 2016;10(5):OC01-OC04. doi:10.7860/JCDR/2016/17631.7713.

45. Willet W. *Nutritional Epidemiology*. Second edi. New York: Oxford University Press; 1998.
46. Sharp DB, Allman-Farinelli M. Feasibility and validity of mobile phones to assess dietary intake. *Nutrition*. 2014;30(11-12):1257-1266. doi:10.1016/j.nut.2014.02.020.
47. Storey KE. A changing landscape: web-based methods for dietary assessment in adolescents. *Curr Opin Clin Nutr Metab Care*. 2015;18(5):437-445. doi:10.1097/MCO.0000000000000198.
48. Gill S, Panda S. Clinical and Translational Report A Smartphone App Reveals Erratic Diurnal Eating Patterns in Humans that Can Be Modulated for Clinical and Translational Report A Smartphone App Reveals Erratic Diurnal Eating Patterns in Humans that Can Be Modulated for H. *Cell Metab*. 2015;22(5):1-10. doi:10.1016/j.cmet.2015.09.005.
49. Zeevi D, Korem T, Zmora N, et al. Personalized Nutrition by Prediction of Glycemic Responses. *Cell*. 2015;163(5):1079-1094. doi:10.1016/j.cell.2015.11.001.
50. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. *Nutr J*. 2012;11(1):109. doi:10.1186/1475-2891-11-109.
51. Scalbert A, Brennan L, Manach C, et al. The food metabolome: a window over dietary exposure. *Am J Clin Nutr*. 2014;99(6):1286-1308. doi:10.3945/ajcn.113.076133.